

Physiology labs for blood (4)

Red cells count & white cells count



المجموعة السابعة

دفعته 33 طب بشري

تلخيص /

هيام الجوبي

أنس العمراني



1st Red cell count

in this test The blood is diluted 200 times and the cells are counted in the counting chamber
Knowing the dilution employed, their number in undiluted blood can easily be calculated.

***reagent** : it is used to dilate blood

the ideal reagent is **formal citrate** , but we can also use **normal saline** as reagent.

***blood sample** : blood collected In EDTA tube

***The Counting Chamber (Improved Neubauer Chamber)**.

also known as **Hemocytometer** , it is the device used to count blood cells under the microscope , it is thick glass contain an H shaped grooves that separate it into 2 platforms . each platform can be used by different sample. Each platform contain counting grid that is seen under the microscope



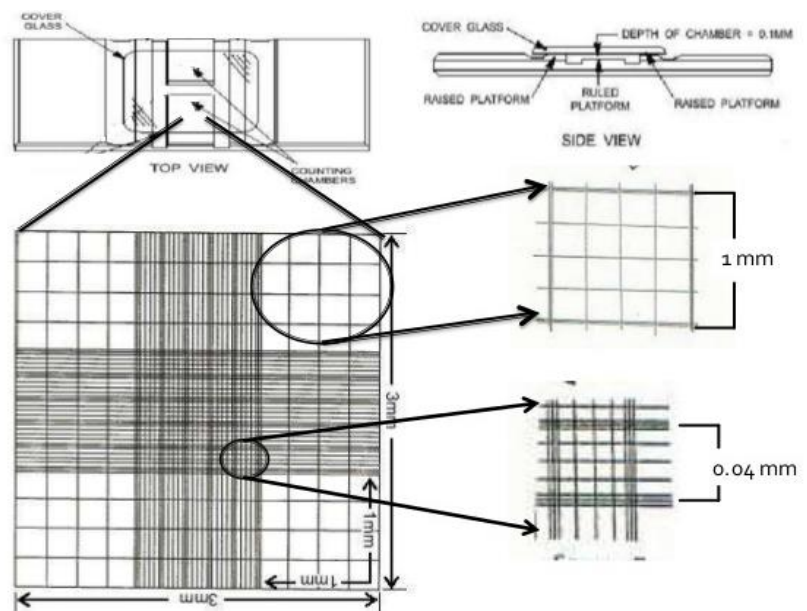
-The counting grids

Each counting grid is divided into 9 large squares

- Of these 9 squares, the 4 large corner squares are lightly etched and each is divided by single lines into 16 medium-sized squares
These 4 large corner squares are employed for counting leukocytes and are, therefore, **called WBC squares**

- The central densely etched large square, **called the RBC square**, is divided into 25 medium-sized squares, Each of these medium squares is set off (separated) from its neighbors by very closely placed double lines or triple lines. These double or triple lines extend in all directions in between all the WBC squares

- Each of the 25 medium squares is further divided into 16 smallest squares by single lines. The red cells are counted in 4 corner groups and one central group of medium squares, each of which has 16 smallest squares, i.e. in a total of 80 smallest squares.



the cover glass

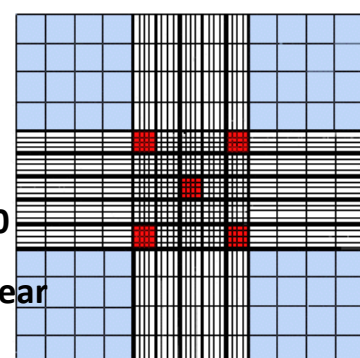
The RBCs are in millions , so to count them easily we have to dilate the blood sample by 1 : 200 dilution factor by the following steps

1- fill the tube with 4 ml of the reagent

2- add 20ul of the blood to the tube , now the blood is diluted by 1 : 200

The high dilution factor makes the WBCs disappear , and only RBCs appear

■ areas of the grid where WBC are counted



■ areas of the grid where RBC are counted

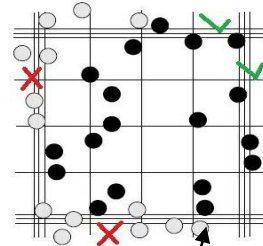
Steps of RBCs count:

- 1- put the cover glass on top of the chamber above the H groove ,use a drop of water to fix it in this position. The cover glass and the chamber should be very clean
- 2- after mixing the diluted blood tube , fill a micropipette or a capillary tube with diluted blood and empty it in the space between the platform and the cover glass.
- 3-put the chamber under the microscope with the x45 lens ,then start counting RBCs in 5 of the medium-sized squares of the central large square following this rules

RULE A : Cells lying within a square are to be counted with that square. And Cells lying on the lines are counted by one of two methods

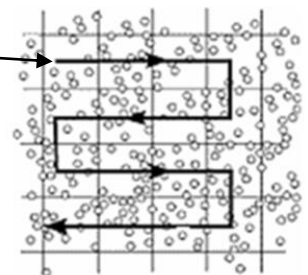
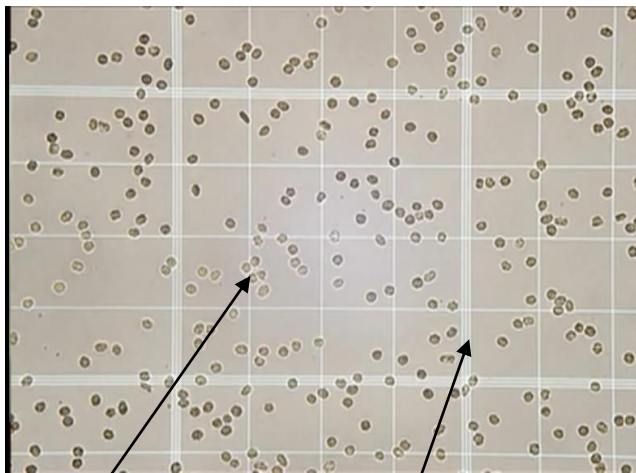
1- "**L**" **method** : counts only the Cells lying on or touching the lower horizontal and left vertical lines

2- "**رقم ٦**" **method** : counts only the Cells lying on or touching the upper horizontal and right vertical lines.



"**رقم ٦**" **method** of the counting rule A /

RULE B : count the squares in zig-zag fashion



counting rule B

This is how the **RBCs** appear under the microscope, they don't overlap each other even In case of polycythemia - note the **double lines** of the medium squares

Calculation

Multiply the total number of RBCs in the 5 of the medium-sized squares x **10,000 "factor"**

Normal range In adult males, it is 5 million/cu mm and in adult females, it is 4.5 million/cu mm.

It Increase in polycythemia it decreases in anemia

*the numbers that we have remember RBCs count are

1- 1:200 the dilution factor

2- 10.000 the Calculation factor

* until now we have studied three tests that can measure RBCs

1- hemoglobin estimation 2- PCV 3- RBCs count

2nd WBCs count

*reagent : contain glacial acetec acid which remove RBCs by destroying them and leave the WBCs intact

*blood sample in EDTA tube

The WBCs sample is diluted by 1:20 factor by the following steps

1- fill the tube with 0.4ml of the reagent

2- add 20ul of the blood to the tube , now the blood is diluted by 1 :20

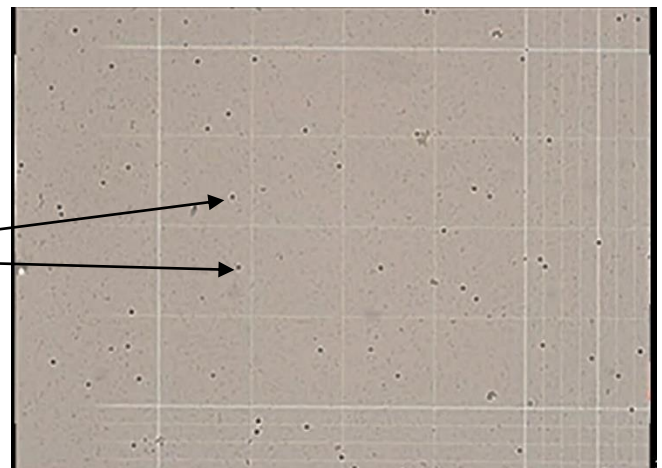
Steps of WBCs count:

1- put the cover glass on top of the chamber above the H groove ,use a drop of water to fix it in this position.

2- after mixing the diluted blood tube , fill a micropipette or a capillary tube with diluted blood and empty it in the space between the platform and the cover glass.

3-put the chamber under the microscope with the x10 lens then start counting WBCs in the 4 large corner squares following the same rules of RBC count.

WBCs appear under the microscope as dots in a white paper or like "stars in the sky "



Calculation

Multiply the total number of WBCs in the 4 large corner squares x 50 "factor"

Normal range in Total WBC count : 4,000 to 11,000/cu mm

it increase in leukocytosis in case of	It decreases in leukopenia in case of
1 acute infection (bacterial) Tonsillitis , meningitis 2 acute hemorrhage : Because the marrow is trying to compensate for the loss by increasing the production of all the cells 3 leukemia 4 physiological : stress - pregnancy - exercise	1 acute infection (viral) Because viruses attack the WBCs 2 chronic infection Like TB 3 aplastic anemia 4 bone marrow infiltration

*the numbers that we have remember in WBCs count are

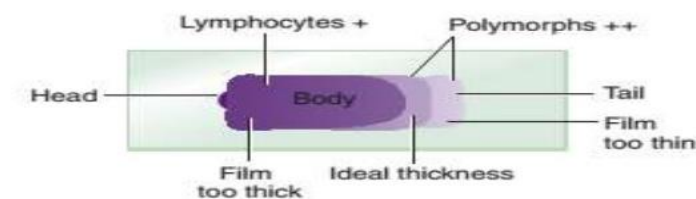
1- 1:20 the dilution factor

2- 50 the Calculation factor

-This test counts only the number of the WBCs so it is called **total WBCs count**

- **The differential WBCs count** counts the percentage of each type of the WBCs and it is done by a blood film

The shape of blood film



To link between **total WBCs count** and **differential WBCs count** we use the words **absolute** and **relative**

for example neutrophilia

Absolute neutrophilia ; if increase total count , and increase the differential count of neutrophils

Relative neutrophilia : normal total count , but increase differential count of neutrophils